

WHAT IS CLAIMED IS:

1           1. An isolated infectious chimeric respiratory  
2 syncytial virus (RSV) comprising a major nucleocapsid (N)  
3 protein, a nucleocapsid phosphoprotein (P), a large polymerase  
4 protein (L), a RNA polymerase elongation factor, and a partial  
5 or complete RSV genome or antigenome of one RSV strain or  
6 subgroup virus combined with a heterologous gene or gene  
7 segment of a different RSV strain or subgroup virus to form a  
8 chimeric RSV genome or antigenome.

1           2. The chimeric RSV of claim 1, wherein the  
2 chimeric genome or antigenome comprises a partial or complete  
3 human RSV genome or antigenome of one RSV subgroup or strain  
4 combined with a heterologous gene or gene segment from a  
5 different, human or non-human RSV subgroup or strain.

1           3. The chimeric RSV of claim 2, wherein the  
2 heterologous gene or gene segment is from a human RSV subgroup  
3 A, human RSV subgroup B, bovine RSV or murine RSV.

1           4. The chimeric RSV of claim 1, wherein the  
2 heterologous gene or gene segment is selected from a NS1, NS2,  
3 N, P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene or gene  
4 segment.

1           5. The chimeric RSV of claim 4, wherein the  
2 heterologous gene or gene segment encodes a RSV F, G or SH  
3 glycoprotein or a cytoplasmic domain, transmembrane domain,  
4 ectodomain or immunogenic epitope thereof.

1           6. The chimeric RSV of claim 1, wherein the  
2 chimeric genome or antigenome comprises a partial or complete  
3 human RSV A subgroup genome or antigenome combined with a  
4 heterologous gene or gene segment from a human RSV B subgroup  
5 virus.

1           7. The chimeric RSV of claim 6, wherein the  
2 heterologous gene or gene segment from human RSV B encodes a

1 RSV F, G or SH glycoprotein or a cytoplasmic domain,  
2 transmembrane domain, ectodomain or immunogenic epitope  
3 thereof.

1 8. The chimeric RSV of claim 6, wherein one or more  
2 human RSV B subgroup glycoprotein genes F, G and SH or a  
3 cytoplasmic domain, transmembrane domain, ectodomain or  
4 immunogenic epitope thereof is substituted within a RSV A  
5 genome or antigenome.

1 9. The chimeric RSV of claim 8, wherein one or both  
2 human RSV B subgroup glycoprotein genes F and G is substituted  
3 to replace one or both counterpart F and G glycoprotein genes  
4 in the RSV A genome or antigenome.

1 10. The chimeric RSV of claim 9, wherein both human  
2 RSV B subgroup glycoprotein genes F and G are substituted to  
3 replace the counterpart F and G glycoprotein genes in the RSV  
4 A genome or antigenome.

1 11. The chimeric RSV of claim 1, wherein a first  
2 heterologous gene or gene segment is substituted to replace a  
3 counterpart gene or gene segment within the partial or  
4 complete RSV genome or antigenome, and a second heterologous  
5 gene or gene segment is added to the partial or complete RSV  
6 genome or antigenome to form the chimeric RSV genome or  
7 antigenome.

1 12. The chimeric RSV of claim 1, wherein the  
2 chimeric genome or antigenome is further modified by one or  
3 more attenuating mutations.

1 13. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least one and up  
3 to a full complement of attenuating mutations present within a  
4 panel of biologically derived mutant RSV strains, said panel  
5 comprising *cpts* RSV 248 (ATCC VR 2450), *cpts* RSV 248/404 (ATCC  
6 VR 2454), *cpts* RSV 248/955 (ATCC VR 2453), *cpts* RSV 530 (ATCC

1 VR 2452), *cpts* RSV 530/1009 (ATCC VR 2451), *cpts* RSV 530/1030  
2 (ATCC VR 2455), RSV B-1 *cp*52/2B5 (ATCC VR 2542), and RSV B-1  
3 *cp*-23 (ATCC VR 2579).

1 14. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least one and up  
3 to a full complement of attenuating mutations specifying a  
4 temperature-sensitive amino acid substitution at Phe<sub>521</sub>, Gln<sub>831</sub>,  
5 Met<sub>1169</sub> or Tyr<sub>1321</sub> in the RSV polymerase gene L, or a  
6 temperature-sensitive nucleotide substitution in the gene-  
7 start sequence of gene M2.

1 15. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least one and up  
3 to a full complement of mutations from cold-passaged  
4 attenuated RSV, said complement of mutations including  
5 mutations specifying an amino acid substitution at Val<sub>267</sub> in  
6 the RSV N gene, Glu<sub>218</sub> or Thr<sub>523</sub> in the RSV F gene, Cys<sub>319</sub> or  
7 His<sub>1690</sub> in the RSV polymerase gene L.

1 16. The chimeric RSV of claim 1, wherein each of  
2 the human RSV B subgroup glycoprotein genes F and G is added  
3 or substituted within a human RSV A genome or antigenome to  
4 form the chimeric genome or antigenome, which is further  
5 modified to incorporate one or more attenuating mutations.

1 17. The chimeric RSV of claim 16, wherein both  
2 human RSV B subgroup glycoprotein genes F and G are  
3 substituted to replace counterpart F and G glycoprotein genes  
4 within an RSV A genome or antigenome to form the chimeric  
5 genome or antigenome, which is further modified to incorporate  
6 attenuating point mutations selected from (i) a panel of  
7 mutations specifying temperature-sensitive amino acid  
8 substitutions at Gln<sub>831</sub> and Tyr<sub>1321</sub> in the RSV polymerase gene  
9 L; (ii) a temperature-sensitive nucleotide substitution in the  
10 gene-start sequence of gene M2; (iii) an attenuating panel of  
11 mutations adopted from cold-passaged RSV specifying amino acid  
12 substitutions Val<sub>267</sub> Ile in the RSV N gene, and Cys<sub>319</sub> to Tyr

13 and His<sub>1690</sub> Tyr in the RSV polymerase gene L; or (iv) a  
14 deletion of the SH gene.

1 18. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome incorporates at least two  
3 attenuating mutations.

1 19. The chimeric RSV of claim 18, wherein the  
2 chimeric genome or antigenome incorporates attenuating  
3 mutations adopted from different biologically derived mutant  
4 RSV strains.

1 20. The chimeric RSV of claim 12, wherein the  
2 chimeric genome or antigenome includes at least one  
3 attenuating mutation stabilized by multiple nucleotide changes  
4 in a codon specifying the mutation.

1 21. The chimeric RSV of claim 1, formulated in a  
2 dose of  $10^3$  to  $10^6$  PFU of attenuated virus.

1 22. The chimeric RSV of claim 1 further comprising  
2 a nucleotide modification specifying a phenotypic change  
3 selected from a change in growth characteristics, attenuation,  
4 temperature-sensitivity, cold-adaptation, plaque size, host-  
5 range restriction, or a change in immunogenicity.

1 23. The chimeric RSV of claim 22, wherein a SH,  
2 NS1, NS2, M2ORF2, or G gene is modified.

1 24. The chimeric RSV of claim 23, wherein the SH,  
2 NS1, NS2, M2ORF2, or G gene is deleted in whole or in part or  
3 expression of the gene is ablated by introduction of one or  
4 more stop codons in an open reading frame of the gene.

1 25. The chimeric RSV of claim 22, wherein the  
2 nucleotide modification comprises a nucleotide deletion,  
3 insertion, substitution, addition or rearrangement of a

4 cis-acting regulatory sequence of a selected RSV gene within  
5 the chimeric RSV genome or antigenome.

1           26. The chimeric RSV of claim 25, wherein the  
2 cis-acting regulatory sequence of the selected RSV gene is  
3 changed to correspond to a heterologous regulatory sequence  
4 comprising a counterpart cis-acting regulatory sequence of the  
5 selected RSV gene from a different RSV subgroup or strain or a  
6 cis-acting regulatory sequence of a different RSV gene.

1           27. The chimeric RSV of claim 25, wherein a gene  
2 end (GE) signal of the NS1 or NS2 gene is modified to  
3 correspond to the GE signal of the RSV N gene.

1           28. The chimeric RSV of claim 22, wherein the  
2 nucleotide modification comprises an insertion, deletion,  
3 substitution, or rearrangement of a translational start site  
4 within the chimeric genome or antigenome.

1           29. The chimeric RSV of claim 28, wherein the  
2 translational start site for a secreted form of the RSV G  
3 glycoprotein is ablated.

1           30. The chimeric RSV of claim 22, wherein the  
2 chimeric genome or antigenome is modified to encode a non-RSV  
3 molecule selected from a cytokine, a T-helper epitope, a  
4 restriction site marker, or a protein of a microbial pathogen  
5 capable of eliciting a protective immune response in a  
6 mammalian host.

1           31. The chimeric RSV of claim 22, which  
2 incorporates a gene or gene segment from parainfluenza virus  
3 (PIV) .

1           32. The chimeric RSV of claim 31, wherein the gene  
2 or gene segment encodes a PIV HN or F glycoprotein.

1           33. The chimeric RSV of claim 32, wherein the gene  
2 segment encodes a cytoplasmic tail, transmembrane domain,  
3 ectodomain or immunogenic epitope of HN or F of PIV1, PIV2, or  
4 PIV3.

1           34. The chimeric RSV of claim 1, wherein the  
2 chimeric genome or antigenome comprises a partial or complete  
3 human RSV genome or antigenome combined with an attenuating,  
4 heterologous gene or gene segment from a bovine or murine RSV.

1           35. The chimeric RSV of claim 1 which is a virus.

1           36. The chimeric RSV of claim 1 which is a subviral  
2 particle.

1           37. A method for stimulating the immune system of  
2 an individual to induce protection against RSV which comprises  
3 administering to the individual an immunologically sufficient  
4 amount of the chimeric RSV of claim 1 combined with a  
5 physiologically acceptable carrier.

1           38. The method of claim 37, wherein the chimeric  
2 RSV is administered in a dose of  $10^3$  to  $10^6$  PFU.

1           39. The method of claim 37, wherein the chimeric  
2 RSV is administered to the upper respiratory tract.

1           40. The method of claim 37, wherein the chimeric  
2 RSV is administered by spray, droplet or aerosol.

1           41. The method of claim 37, wherein the chimeric  
2 RSV is administered to an individual seronegative for  
3 antibodies to RSV or possessing transplacentally acquired  
4 maternal antibodies to RSV.

1           42. The method of claim 37, wherein the chimeric  
2 RSV is a chimera of human RSV A and RSV B which elicits an  
3 immune response against either human RSV A or RSV B.

1           43. The method of claim 37, wherein the chimeric  
2 RSV is a chimera of human RSV A and RSV B which elicits an  
3 immune response against both human RSV A and RSV B.

1           44. The method of claim 37, wherein the chimeric  
2 RSV is a chimera of human RSV A and RSV B which elicits an  
3 immune response against either human RSV A or RSV B and is co-  
4 administered with an immunologically sufficient amount of a  
5 second attenuated RSV capable of eliciting an immune response  
6 against human RSV A or RSV B, whereby an immune response is  
7 elicited against both human RSV A or RSV B.

1           45. The method of claim 44, wherein the chimeric  
2 RSV and second attenuated RSV are administered simultaneously  
3 as a mixture.

1           46. An immunogenic composition to elicit an immune  
2 response against RSV comprising an immunologically sufficient  
3 amount of the chimeric RSV of claim 1 in a physiologically  
4 acceptable carrier.

1           47. The immunogenic composition of claim 46,  
2 formulated in a dose of  $10^3$  to  $10^6$  PFU.

1           48. The immunogenic composition of claim 46,  
2 formulated for administration to the upper respiratory tract  
3 by spray, droplet or aerosol.

1           49. The immunogenic composition of claim 46,  
2 wherein the chimeric RSV is a chimera of human RSV A and RSV B  
3 which elicits an immune response against either human RSV A or  
4 RSV B.

1           50. The immunogenic composition of claim 46,  
2 wherein the chimeric RSV is a chimera of human RSV A and RSV B  
3 which elicits an immune response against both human RSV A and  
4 RSV B.

1           51. The immunogenic composition of claim 46,  
2 wherein the chimeric RSV is a chimera of human RSV A and RSV B  
3 which elicits an immune response against either human RSV A or  
4 RSV B and wherein the composition further comprises an  
5 immunologically sufficient amount of a second attenuated RSV  
6 capable of eliciting an immune response against human RSV A or  
7 RSV B, whereby the composition elicits an immune response  
8 against both human RSV A or RSV B.

1           52. An isolated polynucleotide molecule comprising  
2 a chimeric RSV genome or antigenome which includes a partial  
3 or complete RSV genome or antigenome of one RSV strain or  
4 subgroup virus combined with a heterologous gene or gene  
5 segment of a different RSV strain or subgroup virus.

1           53. The isolated polynucleotide molecule of claim  
2 52, wherein the chimeric genome or antigenome comprises a  
3 partial or complete human RSV genome or antigenome of one RSV  
4 subgroup or strain combined with a heterologous gene or gene  
5 segment from a different, human or non-human RSV subgroup or  
6 strain.

1           54. The isolated polynucleotide molecule of claim  
2 52, wherein the heterologous gene or gene segment is from a  
3 human RSV subgroup A, human RSV subgroup B, bovine RSV, avian  
4 RSV, or murine RSV.

1           55. The isolated polynucleotide molecule of claim  
2 52, wherein the heterologous gene or gene segment encodes a  
3 RSV F, G or SH glycoprotein or a cytoplasmic domain,  
4 transmembrane domain, ectodomain or immunogenic epitope  
5 thereof.

1           56. The isolated polynucleotide molecule of claim  
2 52, wherein the chimeric genome or antigenome comprises a  
3 partial or complete human RSV A subgroup genome or antigenome



4 combined with a heterologous gene or gene segment from a human  
5 RSV B subgroup virus.

1 57. The isolated polynucleotide molecule of claim  
2 52, wherein one or both human RSV B subgroup glycoprotein  
3 genes F and G is substituted to replace one or both  
4 counterpart F and G glycoprotein genes in the RSV A genome or  
5 antigenome.

1 58. The isolated polynucleotide molecule of claim  
2 57, wherein both human RSV B subgroup glycoprotein genes F and  
3 G are substituted to replace the counterpart F and G  
4 glycoprotein genes in the RSV A genome or antigenome.

1 59. The isolated polynucleotide molecule of claim  
2 52, wherein the chimeric genome or antigenome is further  
3 modified by one or more attenuating mutations.

1 60. The isolated polynucleotide molecule of claim  
2 52, wherein both human RSV B subgroup glycoprotein genes F and  
3 G are substituted to replace counterpart F and G glycoprotein  
4 genes within an RSV A genome or antigenome to form the  
5 chimeric genome or antigenome, which is further modified to  
6 incorporate attenuating point mutations selected from (i) a  
7 panel of mutations specifying temperature-sensitive amino acid  
8 substitutions Gln<sub>831</sub> to Leu and Tyr<sub>1321</sub> to Asn in the RSV  
9 polymerase gene L; (ii) a temperature-sensitive nucleotide  
10 substitution in the gene-start sequence of gene M2; (iii) an  
11 attenuating panel of mutations adopted from cold-passaged RSV  
12 specifying amino acid substitutions Val<sub>267</sub> Ile in the RSV N  
13 gene, and Cys<sub>319</sub> to Tyr and His<sub>1690</sub> Tyr in the RSV polymerase  
14 gene L; or (iv) a deletion of the SH gene.

1 61. The isolated polynucleotide molecule of claim  
2 52, further comprising a nucleotide modification specifying a  
3 phenotypic change selected from a change in growth  
4 characteristics, attenuation, temperature-sensitivity,

5 cold-adaptation, plaque size, host-range restriction, or a  
6 change in immunogenicity.

1           62. The isolated polynucleotide molecule of claim  
2 61, wherein a SH, NS1, NS2, M2ORF2, or G gene is modified.

1           63. The isolated polynucleotide molecule of claim  
2 61, wherein the nucleotide modification comprises a nucleotide  
3 deletion, insertion, addition or rearrangement of a cis-acting  
4 regulatory sequence of a selected RSV gene within the chimeric  
5 RSV genome or antigenome.

1           64. A method for producing an infectious attenuated  
2 chimeric RSV particle from one or more isolated polynucleotide  
3 molecules encoding said RSV, comprising:  
4           expressing in a cell or cell-free lysate an  
5 expression vector comprising an isolated polynucleotide  
6 comprising a chimeric RSV genome or antigenome and RSV N, P, L  
7 and RNA polymerase elongation factor proteins.

1           65. The method of claim 64, wherein the chimeric  
2 RSV genome or antigenome and the N, P, L and RNA polymerase  
3 elongation factor proteins are expressed by two or more  
4 different expression vectors.